

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows.

Please replace paragraph [0049] of United States patent application publication no. 20040180378 (the '378 publication) with the following amended paragraph:

[0049] In alternative aspects, the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated saturation mutagenesis (GSSM) (GSSMTM), synthetic ligation reassembly (SLR) and a combination thereof. In some aspects, the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

Please replace paragraph [0257] of the '378 publication with the following amended paragraph:

[0257] Any technique in molecular biology can be used, e.g., random PCR mutagenesis, see, e.g., Rice (1992) Proc. Natl. Acad. Sci. USA 89:5467-5471; or, combinatorial multiple cassette mutagenesis, see, e.g., Crameri (1995) Biotechniques 18:194-196. Alternatively, nucleic acids, e.g., genes, can be reassembled after random, or "stochastic," fragmentation, see, e.g., U.S. Pat. Nos. 6,291,242; 6,287,862; 6,287,861; 5,955,358; 5,830,721; 5,824,514; 5,811,238; 5,605,793. In alternative aspects, modifications, additions or deletions are introduced by error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated saturation mutagenesis (GSSM) (GSSMTM), synthetic ligation reassembly (SLR), recombination, recursive sequence

recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, and/or a combination of these and other methods.

Please replace paragraph [0263] of the '378 publication with the following amended paragraph:

[0263] Non-stochastic, or "directed evolution," methods include, e.g., saturation mutagenesis (GSSM) (GSSMTM), synthetic ligation reassembly (SLR), or a combination thereof are used to modify the nucleic acids of the invention to generate fluorescent polypeptides with new or altered properties (e.g., activity under highly acidic or alkaline conditions, high temperatures, and the like). Polypeptides encoded by the modified nucleic acids can be screened for an activity before testing for fluorescence or other activity. Any testing modality or protocol can be used, e.g., using a capillary array platform. See, e.g., U.S. Pat. Nos. 6,361,974; 6,280,926; 5,939,250.

Please replace paragraph [0264] of the '378 publication with the following amended paragraph:

[0264] Saturation Mutagenesis, or, GSSM GSSMTM

Please replace paragraph [0265] of the '378 publication with the following amended paragraph:

[0265] In one aspect of the invention, non-stochastic gene modification, a "directed evolution process," is used to generate fluorescent polypeptides with new or altered properties. Variations of this method have been termed "gene site-saturation mutagenesis," "site-saturation mutagenesis," "saturation mutagenesis" or simply "GSSM GSSMTM" It can be used in combination with other mutagenization processes. See, e.g., U.S. Pat. Nos. 6,171,820; 6,238,884. In one aspect, GSSM.TM. comprises providing a template polynucleotide and a plurality of oligonucleotides,

wherein each oligonucleotide comprises a sequence homologous to the template polynucleotide, thereby targeting a specific sequence of the template polynucleotide, and a sequence that is a variant of the homologous gene; generating progeny polynucleotides comprising non-stochastic sequence variations by replicating the template polynucleotide with the oligonucleotides, thereby generating polynucleotides comprising homologous gene sequence variations.

Please replace paragraph [0365] of the '378 publication with the following amended paragraph:

[0365] FIG. 5 is a summary of data comparing the properties of exemplary fluorescent polypeptides of the invention DVSAGreen, which is SEQ ID NO:18, encoded by SEQ ID NO:17, and, DVSAcYan, which is SEQ ID NO:8, encoded by SEQ ID NO:7. As noted in FIG. 5, SEQ ID NO:8 (DVSAcYan) is 227 residues in length, has a calculated subunit mass of 25.9 kDa, a total mass of 51.8 kDa, an excitation maximum of 448 (463) nm, an emission maximum of 491 nm, a quantum yield of 0.76, and an extinction coefficient of $18,900 \text{ M}^{-1}\text{cm}^{-1}$. SEQ ID NO:18 (DVSAGreen) is 228 [[253]] residues in length, has a calculated subunit mass of 28.6 kDa, a total mass of 57.3 kDa, an excitation maximum of 487 nm, an emission maximum of 507 nm, a quantum yield of 0.61, and an extinction coefficient of $98,200 \text{ M}^{-1}\text{cm}^{-1}$.